

in a greenhouse. Samples of seed collection Q#026 (deerbrush, collected on the South Fork of Stanislaus River at about 4,800 feet altitude) were treated in boiling water for 1 to 20 minutes. Twelve per cent of these seeds germinated after having been boiled for 20 minutes. Samples of seed collection Q#239 (mountain whitethorn, collected near Strawberry, Stanislaus National Forest, at about 5,400 feet altitude) were treated for 5 to 30 minutes in boiling water. Twenty-five per cent of these seeds germinated after being boiled for 25 minutes, but none germinated after being boiled for 30 minutes. A manuscript, now nearing completion, will analyze statistically the seed germination reactions of several species of *Ceanothus* with respect to seed age, altitude of seed collection, length of exposure to boiling water, and temperature and length of stratification treatment.

Many seeds, even *Ceanothus* seeds, are destroyed in any forest fire, but the above preliminary experiments suggest that seeds of many firetype plants are very durable, long-lived, and sufficiently resistant to high temperatures to escape destruction and to completely revegetate a heavy burn. Thus starts the first stage in a new cycle of forest development.

California Forest and Range Experiment Station,
University of California, Berkeley.

LITERATURE CITED

- JEPSON, WILLIS L. 1925. The life zones. *In* A manual of the flowering plants of California, pp. 4-8. Berkeley, California.
- QUICK, CLARENCE R. 1935. Notes on the germination of *Ceanothus* seeds. *Madroño* 3(3):135-140.
- . 1956. Viable seeds from the duff and soil of sugar pine forests. *Forest Science* 2(1):36-42.

CHROMOSOME NUMBERS OF CALIFORNIA PLANTS, WITH NOTES ON SOME CASES OF CYTOLOGICAL INTEREST

RICHARD SNOW

Meiosis has been examined in microsporocytes of various species of Californian plants, and the resulting chromosome numbers are listed in Table 1. During the course of these observations several meiotic phenomena have been observed that have cytological interest beyond chromosome number. These will be discussed briefly in order to point out some areas possibly worthy of further study.

METHODS. Buds were fixed in 1:3 acetic-alcohol for one to several days and stored in 70 per cent alcohol under refrigeration. After soaking in water for a few minutes the material was softened in 1N HCl at 60°C. for 5-10 minutes, rinsed with water, and the anthers squashed in aceto-orcein or aceto-carmin. The *Fritillaria* material was stained by the Feulgen reaction. Permanent slides were made by Bradley's (1948) method, without removing the coverslip.

TABLE 1. DOCUMENTED CHROMOSOME NUMBERS OF CALIFORNIA PLANTS¹

SPECIES	CHROMO- SOME No. (2n)	LOCALITY AND COLLECTOR
<i>Fritillaria biflora</i> Lindl.*	24+0-8ff	Near Saddle Peak, Santa Monica Mts., Los Angeles County, <i>Lewis</i> in 1958.
<i>Delphinium polycladon</i> Eastw.	16	Rodger's Lake, Tuolumne County, <i>Snow</i> 284.
<i>Ranunculus californicus</i> Benth.	28+f	Road from Bridgeport to Highway 140, Mariposa County, <i>Snow</i> and <i>Wedberg</i> in 1953.
<i>Eschscholtzia mexicana</i> Greene	12	15.3 miles north of Goffs on the road to Lanfair, San Bernardino County, <i>Snow</i> 18.
<i>Thelypodium lasiophyllum</i> (H. & A.) Greene	48	Red Rock Canyon, Kern County, <i>Snow</i> 410.
<i>Isomeris arborea</i> Nutt.*	40	2.7 miles west of Whitewater, Riverside County, <i>Raven</i> 11409. Point Dume, Los Angeles County, <i>Snow</i> (no specimen). Red Rock Canyon, Kern County, <i>Snow</i> 407.
<i>Lupinus superbus</i> Hel. var. <i>elongatus</i> (Greene) C. P. Smith*	48	Matterhorn Canyon, Tuolumne County, <i>Snow</i> 292.
<i>Astragalus Bolanderi</i> Gray	22	Badger Flats, near Huntington Lake, Fresno County, <i>Snow</i> 62.
<i>Astragalus leucopsis</i> (T. & G.) Torr.	22	Point Dume, Los Angeles County, <i>Raven</i> 13857.
<i>Astragalus oocarpus</i> Gray	22	4.6 miles north of Santa Ysabel, San Diego County, <i>Raven</i> and <i>Snow</i> 9557.
<i>Astragalus pomonensis</i> Jones	22	1.7 miles west of Dripping Springs Guard Station, Riverside County, <i>Raven</i> and <i>Snow</i> 9543.
<i>Astragalus crotalariae</i> Benth.	24	Salton Sea State Park, Riverside County, <i>Raven</i> 11484.
<i>Datisca glomerata</i> (Presl) Benth. and Hook.	22	Banning-Idyllwild Road near Grand View Guard Station, Riverside County, <i>Raven</i> and <i>Snow</i> 11115.
<i>Epilobium obcordatum</i> Gray	36	Little Slide Canyon, Mono County, <i>Snow</i> 275.
<i>Oenothera californica</i> Wats. var. <i>californica</i> *	28	11 miles east of Riverside, Riverside County, <i>Snow</i> and <i>Mosquin</i> 318.
<i>Gayophytum racemosum</i> T. & G.	14	Little Slide Canyon, Mono County, <i>Snow</i> 273.
<i>Monardella odoratissima</i> Benth.	42	Little Slide Canyon, Mono County, <i>Snow</i> 272.

¹ Voucher specimens and prepared slides are in the Herbarium of the University of California, Berkeley.

* See discussion in text.

f = fragment chromosome.

SPECIES	CHROMO- SOME No. (2n)	LOCALITY AND COLLECTOR
<i>Datura meteloides</i> DC.*	24	See Table 2.
<i>Plantago insularis</i> Eastw.	8	Near Red Rock Canyon, Kern County, <i>Snow 416</i> .
<i>Pedicularis groenlandica</i> Retz.	16	Pass between Little Slide Canyon and Slide Canyon, Tuolumne County, <i>Snow 276</i> .
<i>Proboscidea Jussieu</i> Keller	30	Putah Creek, west of Davis, Yolo County, <i>Snow 605</i> .
<i>Senecio Fremontii</i> T. & G. var. <i>occidentalis</i> Gray*	40+f	Little Slide Canyon, Mono County, <i>Snow 270</i> .

My thanks are due Dr. Harlan Lewis for the buds of *Fritillaria biflora*, and to Mr. Peter Raven for the material of *Astragalus crocalariae* and for permission to cite his unpublished observations on *Isomeris arborea*.

CYTOLOGICAL NOTES

FRITILLARIA BIFLORA. The fragments in this species are one-third to one-fourth the length of the long arms of chromosomes of the basic complement at anaphase II. It is not certain how constant their number is from plant to plant, since buds from several plants were fixed in the same vial. However, since this species has usually only two to four flowers per plant it is almost certain that each bud in a meiotic stage was derived from a different plant. The number of fragments found in various buds from this collection was 2, 4, 6, or 8. There was also occasionally a smaller variation in number from one PMC to another within the same bud.

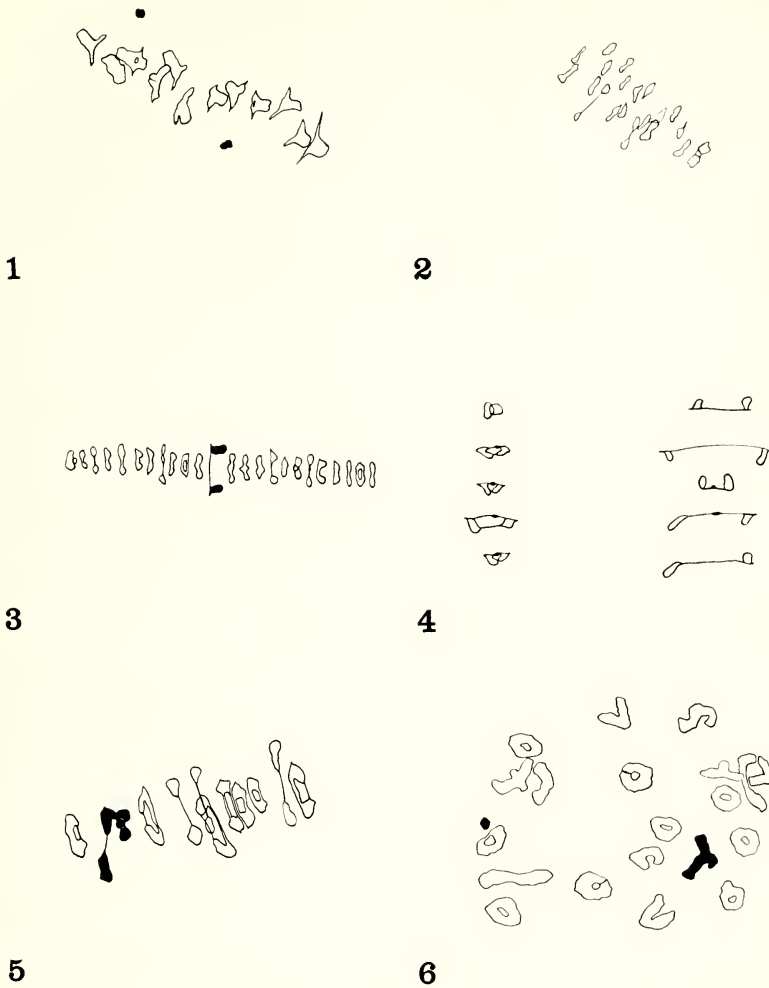
At metaphase I from 1 to 4 "fragment-pairs" have been seen in various buds (fig. 1), and no fragments have been seen at this stage which were not "paired." These dual associations may represent the synapsis of homologous fragments or the division of unpaired fragments. The few mitotic cells on the slides were not clear enough for a definitive answer. The fragments do not congress to the metaphase plate with the other bivalents, but instead usually lie off in the cytoplasm above or below it. Their position at metaphase I may account for their frequent inclusion in the daughter nuclei. However, lagging of the fragments is not infrequent at anaphase. At anaphase I a fragment-pair may separate, one or both halves being included in a nucleus; or the pair may be included, without separation, in one daughter nucleus. As a result of lagging and irregular separation, an anaphase nucleus may receive from none to as many fragments as the PMC contains. Anaphase II distribution is likewise variable. Irregularities of division possibly also occur in mitosis, and may account for the variation in the number of fragments observed in different PMCs of the same bud.

Anaphase observations suggest that the fragments probably possess a centromere, though one which is perhaps less efficient in division than normal. An area which is probably the centromere region appears sub-

terminal in mitotic cells. The preparations were not especially good for study of this feature, however, but certainly the fragments gave no indication of being isobrachial. They do not, therefore, appear to be products of mis-division of a centromere followed by the uniting of sister chromatids to convert a telocentric into an iso-chromosome, as has been found in the triploid *Fritillaria latifolia major* by Darlington (1940; see also Darlington, 1939). Whatever their origin it seems likely that all the fragments observed in the various buds (and hence from several plants) are the result of a single primary event of the past, both because their similar form and because the formation of nuclei with extra fragments as a result of non-disjunction offer cytological evidence of a means for their increase. Pollen grains with fragments are no doubt functional at least occasionally, if we may extrapolate from the results of Kayano (1956), who found almost a 1:1 ratio of $2n:2n+f$ plants in the cross *Lilium callosum* $2n$ ♀ \times $2n+f$ ♂. In this case the fragment appears to be a telocentric from an originally acrocentric supernumerary. As in *F. biflora*, however, no pairing of the fragment with the basic set occurred at metaphase I, though several fragments could pair among themselves.

The western American species *F. lanceolata*, *F. pudica*, *F. recurva* and *F. folcata* are reported to have fragment chromosomes (Beetle, 1944; La Cour, 1951). In addition, Darlington and Wylie (1955) list several Old World species, and also the Californian species *F. pluriflora*, as having B chromosomes; these may be the same type as those present in *F. biflora*. It is possible that such chromosomes are present throughout the genus, in some species frequent, in others rarer. What their role may be in the population dynamics and evolution of the species remains to be determined.

ISOMERIS ARBOREA. This plant has enjoyed a measure of fame since Billings (1937) reported it to be haploid with a highly anomalous embryology. Since then several workers have reinvestigated the embryology and found it to be essentially normal, although with certain peculiar features (Maheswari and Kahn, 1953; Sachar, 1956). The chromosome situation, however, has not yet been clarified. Observations of meiosis made by Mr. Peter Raven of a plant growing near Whitewater, Riverside County, and by the author of one plant from Point Dume, Los Angeles County, and of another from Red Rock Canyon, Kern County, have shown the species to be a diploid with 20 pairs of chromosomes (fig. 2). The chromosomes are small and difficult to stain well, and buds in meiosis are found rather infrequently, so that it is not favorable material cytologically. However, these observations have shown a normal meiotic sequence from diakinesis through telophase II. Further, my examination of pachytene showed two paired strands in clear cells, and in a few instances where the synapsed chromosomes were more widely separated than usual they could be seen to have a similar chromomere pattern. From the figures published by Billings, it seems clear he observed normal meta-



FIGS. 1-6. Chromosome plates. 1, *Fritillaria biflora*: 12 bivalents plus 2 "fragment-pairs"; 2, *Isomeris arborea*: 20 bivalents; 3, *Lupinus superbus* var. *elongatus*: 24 bivalents, the largest with unequal arms; 4, *Lupinus superbus* var. *elongatus*: left, five A bivalents at metaphase or early anaphase, right, five A bivalents at anaphase; 5, *Datura meteloides*: 10 bivalents plus a chain of 4 chromosomes; 6, *Senecio Fremontii* var. *occidentalis*: 20 bivalents plus 1 fragment.

(Fig. 1, $\times 358$; Fig. 2, $\times 952$; Figs. 3-6, $\times 1071$.)

phases and anaphases in both of the meiotic divisions, but as he was convinced that only univalents were present at diakinesis, he interpreted these as equational separations of univalents.

Isomeris arborea must therefore be returned to the category of rather ordinary plants.

LUPINUS SUPERBUS var. ELONGATUS. At metaphase 24 bivalents are present. One of them is conspicuously larger than the rest, and I designate it the A bivalent (fig. 3). In the pollen mother cells from one inflorescence this bivalent was unequal, that is, one chromosome was shorter than the other. Judging from metaphase appearances the A chromosomes are probably acrocentric, and regularly form a chiasma in the short arm. In about ten to twenty per cent of the cells the longer (unequal) arms also show evidence of a chiasma. Figure 4, right, shows the appearance of 5 A bivalents with a chiasma in the short arm; figure 4, left, 5 with chiasmata in both arms. It is possible that the A chromosomes usually form a chiasma in each arm, and that early terminalization in the longer arms releases them from their association. This is suggested by the fact that clear one-chiasma bivalents appear further along in anaphase separation than those with two chiasmata.

White (1954) has given a diagram showing the various types of meiotic segregations which have been observed or are presumed to occur with unequal bivalents of orthopteroïd insects. The segregation at the first division will depend upon three factors: the position of the centromere, the position of the chiasmata, and the position of the inequalities. His diagram is based on two assumptions: that the extra segment is terminal rather than interstitial, and that only one chiasma is formed in the unequal bivalents. White has based these two assumptions on the evidence presently available from grasshoppers and phasmids. In *Lupinus superbus* we have what appears to be an example of an interstitial inequality in an unequal bivalent where chiasmata are formed in each arm, a situation for which White had no examples. The evidence for the existence of more than one chiasma in the A bivalent is unequivocal: two-chiasmata bivalents have been seen in many metaphase cells. The evidence for the interstitial position of the inequality is less direct. It depends on the fact that only reductional separations of the inequality have been observed in both one-chiasma and two-chiasmata A bivalents. Should a chiasma be formed in the long arm proximal to a terminal inequality, then an equational separation would result, with one chromatid of the anaphase chromosome longer than the other. Such equational separations were never observed in about fifty cells analyzed, although it must be admitted that equational separations might not be readily detected, since the chromosomes are rather small. Furthermore, only terminal junctions have been found in the unequal arm of the A bivalent at metaphase, while a chiasma proximal to an inequality would be expected to remain interstitial at this stage. It thus appears that reductional separation of the inequality is the rule, and hence that it occupies an interstitial position in the chromosome arm. Whether it is a duplication, a deficiency, or a heterochromatic region (as is so often the case in insects), has not been determined.

Two other chromosomal types might be expected in the population, namely the corresponding homozygotes. The size difference between the A chromosomes is great enough so that there should be no difficulty dis-

tinguishing them. First metaphase was studied in another inflorescence (and hence probably from another plant). In this case both members of the A bivalent appeared about the same size, and were as large as the larger member of the heterozygous plant. If the inequality is a deficiency, the homozygote for the smaller member may not be viable. A much larger sample of the population would be desirable in order to determine the prevalence of the three types, and to compare their frequencies with the expectation based on the Hardy-Weinberg formula.

OENOTHERA CALIFORNICA var. *CALIFORNICA*. The plant examined of this collection was a tetraploid ($n=14$). A varying number of pairs and rings of four chromosomes was found at diakinesis and metaphase, the maximum seen being six rings of 4 plus 2 pairs. The pairing observed suggests that this species was derived from a diploid form which formed 7 bivalents. In *Oe. californica* var. *glabrata*, Lewis et al. (1958) reported two associations of 8 chromosomes, indicative that the diploid form might have been a structural heterozygote. These observations on *Oe. californica* (subgenus *Anogra*) parallel those of Hagen (1950) on two tetraploid species of the subgenus *Raimannia*, who found only pairs or rings of 4 in *Oe. tetragona*, but long chains (of up to 14 chromosomes) and univalents in *Oe. speciosa* var. *Childsii*. In this latter species, diploids are known which form one or two rings of 4, or 7 bivalents.

As is usual with multivalent configurations in the Onagraceae, adjacent chromosomes were regularly segregated to opposite poles at anaphase, thus supporting Garber's (1954) suggestion that if a diploid species shows directed segregation of chromosomes from interchange configurations, the autotetraploid will likewise show directed segregations of the quadrivalents. These observations coincide with those of Catcheside (personal communication to Garber, l.c.) that "*Oenothera* tetraploids from structurally homozygous diploids show almost regularly zigzag orientation of the rings of four." As a consequence of such regular segregation and the lack of lagging chromosomes, fertility in this tetraploid should be quite high.

DATURA METELOIDES. Ten wild plants of this species have been examined and five have been found to be heterozygous for a reciprocal translocation (table 2). A sixth (from Caliente Creek) suggests by frequent univalent formation that it may also be heterozygous for a structural change which is unidentified at present. The plants are apparently not all heterozygous for the same interchange, because a plant from Isabella Reservoir and plants from Putah Creek (*Snow* 322-5, 324-5) characteristically formed rings of four at metaphase, while in the Yucaipa plant (11076B) only chains of four were observed. In a third Putah Creek plant (*Snow* 322-1) variable configurations were formed at metaphase. Usually the groups of four chromosomes appeared as a branched chain (fig. 5). Sometimes one of the pairs of chromosomes of the configuration had a chiasma in each arm, so that the association resembled a kite with the

TABLE 2. LOCALITIES AND METAPHASE ASSOCIATIONS OBSERVED IN WILD INDIVIDUALS OF *DATURA METELOIDES*

LOCALITY AND COLLECTOR	METAPHASE ASSOCIATIONS
Near Ensenada, Baja California, Mexico, <i>Snow</i> (no specimen).	12(2)
Near Yucaipa, Riverside County, <i>Raven</i> and <i>Snow</i> 11076A.	12(2)
Near Yucaipa, Riverside County, <i>Raven</i> and <i>Snow</i> 11076B.	10(2) + (4c)
Caliente Creek, Kern County, <i>Snow</i> 248.	12(2) or 11(2) + 2(1)
Isabella Reservoir, Kern County, <i>Snow</i> 253. (coll. 6/18/54).	10(2) + (4r)
Isabella Reservoir, Kern County, <i>Snow</i> 253A. (coll. 7/30/57).	12(2)
Putah Creek, south of Davis, Yolo County, <i>Snow</i> 322-1.	10(2) + (4c) or 10(2) + (4r)
Putah Creek, south of Davis, Yolo County, <i>Snow</i> 322-5.	10(2) + (4r)
Putah Creek, south of Davis, Yolo County, <i>Snow</i> 322-8.	12(2)
Putah Creek, west of Davis, Yolo County, <i>Snow</i> 324-5.	10(2) + (4r)

(2) = bivalent

(1) = univalent

(4c) = chain of four

(4r) = ring of four

tail coming off one side. Other configurations observed, much less frequently, were a "T" with a univalent, a non-disjunctional ring of 4, and 12 bivalents.

Staiger (1955) has found interchanges in natural populations of the mollusc *Purpura lapillus* which lead to metaphase configurations similar to those which I have found in one plant (*Snow* 322-1). He has shown how they may be accounted for by the interchange of short end pieces of metacentric chromosomes plus variations in chiasma formation. The same scheme can account for the configurations in this plant of *Datura meteloides*. The translocations in other plants probably represent larger exchanges of chromosome end segments.

Four chromosomal arrangements have been found in this species by Satina (1953), and one of them (type I) has been compared to the standard race of *Datura stramonium*. The other three arrangements presumably differ from type I by reciprocal translocations. The type I arrangement has been found in twenty-eight races. Unfortunately the origin of none of these races is cited in the paper, so that the geographical distribution of this type is unknown. Furthermore, the existence in nature of individuals heterozygous for reciprocal translocations was not reported for *D. meteloides*, and apparently has not been for any other species of *Datura*, even though individuals of the same species may be homozygous for different chromosome arrangements while members of different species invariably are.

Study of *D. meteloides* is being continued with the view of discovering the role translocations may play in the populations.

SENECIO FREMONTII var. *occidentalis*. The extra chromosome in this

material is a small fragment about one-fourth the length of an arm of the longer chromosomes of the complement at diakinesis. At this stage it is often found lying in the proximity of a bivalent with widely diverging arms, one of which appears shorter than the other by about the size of the fragment. The fragment may even appear attached to this arm by strands of stainable material. With about an equal frequency, however, it is found completely free of this readily-recognizable bivalent (fig. 6).

Stages later than diakinesis were not favorable for study in these preparations. In a few metaphase cells which could be analyzed, the fragment was attached to a bivalent, probably a continuation of a diakinetically associated association which was so often observed. The fragment was included in one of the daughter nuclei in all instances save one. In this one instance, out of about fifty cells in telophase examined, the fragment had been excluded and was dividing.

Department of Genetics,
University of California, Davis

LITERATURE CITED

- BEETLE, D. E. 1944. A monograph of the North American species of *Fritillaria*. *Madroño* 7:133-159.
- BILLINGS, F. H. 1937. Some new features in the reproductive cytology of Angiosperms, illustrated by *Isomeris arborea*. *New Phytol.* 36:301-326.
- BRADLEY, M. V. 1948. A method for making aceto-carmin slides permanent without removal of the cover slip. *Stain Tech.* 23:41-44.
- CLELAND, R. E. (ed.) 1950. *Studies in Oenothera cytogenetics and phylogeny*. Indiana Univ. Publ. Sci. Ser. No. 16. 348 pp.
- DARLINGTON, C. D. 1939. Misdivision and the genetics of the centromere. *Jour. Genet.* 37:341-364.
- . 1940. The origin of iso-chromosomes. *Jour. Genet.* 39:351-361.
- and A. P. Wylie. 1955. *Chromosome Atlas of Flowering Plants*. George Allen and Unwin, Ltd. London.
- GARBER, E. D. 1954. Cytotaxonomic studies in the genus *Sorghum*. III. The polyploid species of the subgenera *Parasorghum* and *Stiposorghum*. *Bot. Gaz.* 115:336-342.
- HAGEN, C. W., JR. 1950. A contribution to the cytogenetics of the genus *Oenothera* with special reference to certain forms from South America, in Cleland, 1950, pp. 305-348.
- KAYANO, H. 1956. Cytogenetic studies in *Lilium callosum*. II. Preferential segregation of a supernumerary chromosome. *Mem. Fac. Sci., Kyushu Univ., Ser. E*, 2:53-60.
- LA COUR, L. F. 1951. Heterochromatin and the organization of nucleoli in plants. *Heredity* 5:37-50.
- LEWIS, H., P. H. RAVEN, C. S. VENKATESH, and H. L. WEDBERG. 1958. Observations of meiotic chromosomes in the Onagraceae. *El Aliso* 4:73-86.
- MAHESHWARI, P. and R. KAHN. 1953. Development of the embryo sac, endosperm, and embryo in *Isomeris arborea*—a reinvestigation. *Phytomorphology* 3:446-459.
- SACHAR, R. C. 1956. The embryology of *Isomeris*—a reinvestigation. *Phytomorphology* 6:346-363.
- SATINA, S. 1953. Chromosome end arrangements in *Datura innoxia*, *D. meteloides*, and *D. metel*. *Am. Jour. Bot.* 40:638-646.
- STAIGER, H. 1955. Reziproke translokationen in natürlichen populationen von *Purpurella lapillus* (Prosobranchia). *Chromosoma* 7:181-197.
- WHITE, M. J. D. 1954. *Animal Cytology and Evolution*. 2nd edition. Cambridge University Press.